"A Comparative Analysis of *Cannabis sativa* and *Cannabis indica* for Phytoremediation of Lead and Cadmium in Partapur Industrial Area,meerut (U.P)

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A SYNOPSIS

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1. INTRODUCTION

The contamination of soil and water by heavy metals represents a major environmental challenge in today's world (Chhotu and Fulekar, 2009). Various forms of pollution continue to rise due to increasing human activities, which ultimately introduce these contaminants into the environment. Contaminated soil and water pose serious health risks to both humans and animals. Heavy metals are a major source of such pollutants (Tangahu et al., 2011).They can enter the food chain through plants and accumulate in animals via bio magnification (Sarwar et al., 2010). The concentration of heavy metals ranges from 1 to 100,000 mg/kg, and they are classified as toxic (Blaylock and Huang, 2000). Cadmium (Cd) and Lead (Pb) have the potential to induce the formation of Reactive Oxygen Species (ROS), which can lead to harm to cellular components, including DNA and proteins (Stanley et al., 2018).

Lead (Pb) and cadmium (Cd) are among the most toxic heavy metals, posing severe risks to both ecological and human health due to their persistence, bioaccumulation, and non-biodegradability (Alloway, 2013). Western Uttar Pradesh, with its expanding industrial base, has witnessed alarming levels of these pollutants, necessitating innovative and sustainable remediation approaches.

Phytoremediation is an environmentally friendly and affordable approach that utilizes plants to eliminate, secure, or neutralize environmental contaminants. This method has emerged as a promising alternative to traditional remediation techniques (Ali et al., 2013). Plants that can tolerate and accumulate heavy metals play a crucial role in detoxifying contaminated sites. Among potential phytoremediators, *Cannabis* species—*Cannabis sativa* and *Cannabis indica*—have emerged as promising candidates due to their rapid growth, high biomass production, and demonstrated capacity for metal accumulation (Linger et al., 2005). These species have shown differential capabilities in absorbing and tolerating heavy metals, making them suitable subjects for comparative analysis. Recent studies have further highlighted the metal-tolerance mechanisms of *Cannabis sativa*, including root morphology adaptations and enhancedproduction of phytochelatins and other metal-binding proteins (Ahmad et al., 2020; Angelova et al., 2021).

Despite their shared genus, *Cannabis sativa* and *Cannabis indica* exhibit notable physiological and morphological differences, which may influence their effectiveness in metal uptake and tolerance. Comparative studies on their phytoremediation capabilities remain limited, especially under localized conditions.

This study aims to evaluate and compare the phytoremediation potential of *Cannabis sativa* and *Cannabis indica* for lead and cadmium in contaminated soils of Western Uttar Pradesh. The region's unique agro-climatic conditions and the widespread occurrence of heavy metal pollution necessitate localized solutions. By understanding the mechanisms and efficiencies of these two cannabis species, the research intends to contribute to the development of tailored phytoremediation strategies for sustainable environmental management.

TAXONOMIC CLASSIFICATION OF *Cannabis sp.*

Kingdom: Plantae

Clade: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Sub-class: Hamamelididae

Order: Urticales

Family: Cannabaceae

Genus: *Cannabis* L.

Species: *sativa* L.

Species: *indica* L.

2. REVIEW OF LITERATURE

Phytoremediation: Phytoremediation is a plant-based technology employed either raw or genetically modified plant species for restoring contaminated land and water sources (Harveyetal.2002;Karenlampietal.2000;Purvis2000;Arthuretal.2005;Parmar and Singh 2015).This technique is cost-effective and sustainable compared to traditional methods like excavation and chemical treatments (Ghosh & Singh, 2005).

The sources of heavy metals in agricultural lands are from mining, drilling, warfare activities, sewage sludge, tannery, electroplating, batteries, pesticides and fertilizers (Wei and Yang 2010). The success of phytoremediation depends on the plant species, site conditions, and the type of pollutant present, making it essential to carefully select appropriate plants for each scenario (Pilon-Smits,2005).Despite its potential, phytoremediation is limited by factors such as the slow growth rate of plants and the depth of root penetration, which restrict its applicability in severely contaminated or deep soil sites (Yoon et al., 2006).

Phytoremediation includes several plant-based strategies:

1. **Rhizofiltration**: Using plant roots to clean aquatic environments(Dushenkov et al., 2002)
2. **Phytostabilization**: Stabilizing contaminated soil to reduce erosion or leaching(Berti and Cunningham 2000).
3. **Phytovolatilization**: Extracting and releasing pollutants into the atmosphere(Zayed et al. 2000).
4. **Phytoextraction**: Absorbing contaminants into plant tissues, particularly in harvestable shoots (Blaylock and Huang, 2000).

Phytoextraction is especially effective for addressing metal contamination as it involves the uptake and storage of metals in above-ground plant parts for subsequent removal (Blaylock and Huang, 2000). Similarly phytostabilization helps control the spread of contaminants, as seen in successful applications like using *Festuca rubra* for stabilizing erosion-prone, metal-contaminated soils (Lasat,2000).Effective plant selection for phytoremediation depends on

criteria like swift development, significant biomass yield, pollutant tolerance, and their capacity to uptake and store metals in harvestable tissues (Ali et al., 2013; Pilon-Smits, 2005).

Hyperaccumulator plants, which can withstand and store substantial concentrations of heavy metals within their tissues, are essential contributors to the phytoremediation process (Chaney et al., 2007). Hyperaccumulators can absorb and tolerate exceptionally high metal concentrations, often exceeding 0.1% of their dry weight (VanderEnt et al., 2013; Rascio & Navari-Izzo, 2011).

While hyperaccumulators hold significant potential for phytoremediation, efforts continue to identify plants that balance high accumulation rates, fast growth, and biomass production for broader practical applications.

Heavy Metals Selected for Study:

LEAD

Lead, a chemical element classified in Group 14 (formerly Group 4) and located in the sixth row of the periodic table, is designated by the atomic number 82 (Greenwood & Earnshaw, 2012; Reimann & de Caritat, 2021). It has an atomic mass of 207.2, a density of 11.4 g/cm³, a melting point of 327.4°C, and a boiling point of 1725°C (Lide, 2005; Emsley, 2011). In its natural state, lead is a bluish-grey metal often found in mineral forms combined with other elements such as sulfur (e.g.PbS, galena; PbSO□, anglesite) or oxygen (e.g.,PbCO□,cerussite) (Krishnamurthy & Gupta,2015;Chowdhury et al.,2016). Globally, the average lead concentration in surface soils is approximately 32 mg/kg, with a range of 10 to 67 mg/kg (Kabata-Pendias and Pendias, 2001).Lead stands as the fifth most widely produced industrial metal, coming after, Zinc, Iron, Aluminum, and Copper (International Lead and Zinc Study Group, 2021; Rauch & Graedel, 2007).

Lead contamination primarily arises from vehicle exhaust and industrial emissions, particularly from factories utilizing lead (Berglund et al., 2000; Li et al., 2001). Additional sources include waste generated by battery manufacturing plants, mining

Operations, smelting of lead ores, and metal electroplating processes (Alloway, 2013; Kumar et al., 2015; Zhao et al., 2019). Other contributors are the production of fertilizers and the use of lead compounds as additives in gasoline (Bakir et al., 2006; Landrigan et al., 2002). In agriculture, lead compounds like lead arsenate have been employed as pesticides which, when used excessively can lead to significant soil contamination (Howard, 2003; Goyer, 2001).

Sunflower and maize are two species that can tolerate soils with lead concentrations of up to 16,000 mg Pb/kg (Spirochova et al., 2003).Studies have shown that Piptatherum miliaceum, commonly known as smilo grass, can be used to mitigate the effects of lead contamination in soil, which can have concentrations ranging from 300 to 1,500 mg/kg (Garcia et al., 2004). Thlaspi praecox is known to have the ability to uptake a large amount of Pb from soil which has concentrations of 67,940 mg Pb kg-1 (Mikus et al., 2005).

CADMIUM

Cadmium positioned at the end of the second row of transition metals, has an atomic number of 48 and an atomic weight of 112.4 (Emsley, 2001; Greenwood & Earnshaw, 2012). It has a density of 8.65 g/cm^3, a melting point of 320.9°C, and a boiling point of 765°C (Lide, 2000; Barin, 2003). Alongside mercury (Hg) and lead (Pb), cadmium significantly contributes to soil contamination and has no beneficial role in plant growth (Alloway, 2004; Satarug et al., 2003).

Cadmium contamination primarily arises from various anthropogenic sources, including the mining and smelting of cadmium ores, the disposal of electronic waste, the use of phosphate fertilizers, and the combustion of fossil fuels (Zhang et al., 2003). Industrial processes such as the production of batteries, pigments, and metal plating also contribute significantly to cadmium release into the environment (Bhattacharya et al., 2003; He et al., 2008). Additionally, municipal waste incineration and the burning of coal further exacerbate cadmium pollution in soils and waters (Kabata-Pendias & Pendias, 2001; Wang et al., 2012).

Cadmium is often found as an impurity and a by-product in the production of fertilizers, detergents, and petroleum (Williams et al., 2002; Selim et al., 2007).Normally Cd is accumulated in the root system and it is transported to the shoot system (Yoon et al., 2006).

One study found that *Cannabis sativa* can effectively absorb cadmium from contaminated soils, thanks to its strong root system and efficient metal uptake mechanisms (Chaney et al., 2007; Ghosh et al., 2003). Another investigation highlighted the potential of *Cannabis indica* in phytoremediation, where it was demonstrated to tolerate and accumulate cadmium in its tissues, making it suitable for use in polluted environments (Zheng et al., 2007; Rizwan et al., 2013).

Cannabis sp.

Cannabis, a genus within the Cannabaceae family is most commonly associated with the species *Cannabis sativa* (Clarke and Merlin 2013).The genus comprises three species: *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*, each originating from different geographic regions and exhibiting unique phenotypes (Hillig, 2005). These species are characterized by their rapid growth, high biomass production, and deep, fibrous root systems, which make them particularly suitable for phytoremediation (Linger etal.,2002). Among these, *Cannabis sativa* is the most extensively studied and cultivated due to its wide-ranging applications in industrial, medicinal, and environmental contexts (Andre et al., 2016). Throughout the domestication process wild ancestral populations of Cannabis have undergone selective pressures leading to the development of the diverse varieties seen today (Clarke & Merlin, 2013).

Cannabis sativa has shown significant potential for heavy metal phytoremediation due to its ability to accumulate toxic metals like cadmium, lead, and chromium in its tissues (Ahmad et al., 2015). This accumulation is facilitated by its high tolerance to metal-induced oxidative stress, aided by the production of secondary metabolites like flavonoids and terpenoids that act as antioxidants (Mishra et al., 2016).

Cannabis species are advantageous because they can grow in diverse environmental conditions, including contaminated soils where other crops might fail (Khan et al., 2013). Their economic value as a source of fiber, oil, and biofuel also adds a dual benefit to remediation efforts, making the cleanup process more cost-effective (Citterio et al., 2003).

Drug-type cannabis plants were categorized into two primary groups commonly referred to as "Indica" and "Sativa" (Small & Cronquist, 2002; Hillig,2005).This classification became widely recognized after highlighting their morphological differences and geographic origins. *C. sativa* typically grows taller, with heights reaching up to 5-6 meters, and has a slender structure. In contrast, *C. indica* is shorter, bushier, and rarely exceeds 2-3 meters in height (Hillig & Mahlberg, 2004). *C. sativa* features thin, long leaflets, while *C. indica* has broader and shorter leaflets (Small & Cronquist, 2002). The extensive root system of *C. sativa* provides a greater surface area for pollutant absorption, while the relatively compact roots of *C. indica* are better suited for phytostabilization in smaller, localized areas (Linger et al., 2002). Studies have shown that *C.sativa* can also be used in rhizofiltration to remove heavy metals from contaminated water due to its vigorous root growth (Khan et al., 2013). *C. indica* plays a prominent role in phytostabilization, where it immobilizes contaminants in the soil, reducing their mobility and bioavailability (Gioria et al., 2007).Due to its higher production of secondary metabolites like terpenoids and flavonoids, it shows greater tolerance to oxidative stress caused by toxic metals, which is essential for long-term remediation in heavily polluted areas (Mishra et al., 2016).

Linnaeus's *C. sativa* type specimen was a fiber-type hemp plant rather than a drug-type variety, making the modern application of the term "Sativa" to drug-type plants technically inaccurate. Ideally, this term should be reserved for low-THC fiber-type hemp (Clarke & Merlin, 2013; Hillig & Mahlberg, 2004). Conversely, Lamarck identified *C.indica* to describe drug-type plants originating from India, along with their descendants in Southeast Asia and Africa—plants that today are often incorrectly referred to as "Sativa" (Small & Cronquist, 2002; Hillig, 2005).

Chemically, "Sativa" plants typically produce higher levels of THC than CBD and have a terpenoid profile that emits herbal or sweet aromas. On the other hand, "Indica" plants tend to have a more balanced THC-to-CBD ratio, often closer to 1:1, and their terpenoids contribute to a pungent or skunky scent (Clarke & Merlin, 2013; Hillig, 2005).

In 2001, P. Linger and colleagues utilized atomic absorption spectroscopy to examine the fiber quality of industrial hemp. They concluded that factors like heavy metal levels in soil significantly influence fiber properties (Linger et al., 2002). Two years later, in 2003, Italian researchers Sandra Citterio et al. studied heavy metal accumulation in *Cannabis sativa* roots, revealing that soil pollution can impact the plant's THC content (Citterio et al., 2003). In the same year, researchers B. Kos and D. Lesten analyzed the concentrations of lead, zinc, and cadmium in *Cannabis sativa*, examining their removal from soil using biodegradable chelators, permeable barriers, and induced phytoextraction techniques (Kos & Lesten, 2003).

By 2005, P. Linger et al. conducted further studies on the growth and cadmium absorption of *Cannabis sativa* grown in heavy metal-contaminated soil. They found that germination remained unaffected at cadmium concentrations of up to 72 mg/kg. However, while roots initially accumulated high levels of cadmium—up to 830 mg/kg—this concentration decreased as the plant matured (Linger et al., 2005).

In 2012, Bruci et al. employed GC-MS to assess the Cannabinoids composition of *Cannabis sativa* cultivated in Albania. Their research highlighted that Cannabinoids concentrations vary significantly between plant organs, depending on factors such as maturity and sexual differentiation. THC levels in different Albanian regions ranged from 1.07% to 12.13% (Bruci et al., 2012).

In 2023, Fidan et al. reported that *Cannabis sativa* grown in diverse locations exhibited variations in morphology and phytochemical profiles. These differences have contributed to the classification of *Cannabis sativa* into two major categories: Industrial Cannabis (fiber-type hemp) and Medical Cannabis (Fidan et al., 2023). In the same year, Marabesi et al. investigated the effects of cadmium exposure on industrial hemp cultivated for Cannabinoids production. They warned of the health risks posed by cadmium accumulation in smoke-producing plants like hemp and tobacco, as cadmium is a Group 1 carcinogen. Their findings indicated that cadmium accumulation in *Cannabis* varies by plant variety (Marabesi et al., 2023).

3 RESEARCHGAP

Among various plants, *Cannabis sativa* and *Cannabis indica* are recognized for their ability to accumulate heavy metals like Lead (Pb) and Cadmium (Cd). However, several gaps in the existing literature highlight the need for further investigation:

Regional Focus:

- Most studies on the phytoremediation potential of *Cannabis* species have been conducted in controlled environments or in regions with different climatic and soil conditions.
- The specific conditions of Western Uttar Pradesh with its unique industrial and agricultural pollution profile remain underexplored.

Species-Specific Analysis:

- Limited comparative studies exist on the differential uptake and tolerance capacities of *Cannabis sativa* and *Cannabis indica*.
- The unique traits of these species including root morphology, biomass production, and metal accumulation mechanisms are not well-documented in the context of Lead (Pb) and Cadmium (Cd) remediation.

Metal-Specific Mechanisms:

- While general studies have reported the ability of cannabis to accumulate heavy metals, the specific pathways and mechanisms involved in Lead (Pb) and Cadmium (Cd) uptake, translocation, and sequestration remain inadequately understood.
- The influence of soil properties such as pH, organic matter content, and heavy metal speciation, on the phytoremediation efficiency of these species is poorly addressed.

Field Studies vs. Laboratory Experiments:

- Much of the research on cannabis phytoremediation is limited to laboratory experiments with minimal field validation.

- Real-world applications particularly in contaminated sites of Western U.P. require extensive field studies to evaluate the practical feasibility and scalability of this approach.

Impact of Heavy Metals on Plant Growth:

- There is insufficient data on how varying levels of Lead (Pb) and Cadmium (Cd) contamination impact the growth, biomass, and Cannabinoids content of *Cannabis sativa* and *Cannabis indica*.
- Understanding these impacts is crucial for balancing phytoremediation efficiency with potential industrial or medicinal uses of the plants.

Policy and Regulation Context:

- Despite the growing interest in cannabis-based phytoremediation, regulatory challenges surrounding the cultivation of cannabis plants especially in India limit its widespread adoption.
- There is a lack of research linking scientific findings with policy frameworks to promote cannabis phytoremediation as a viable strategy in contaminated regions.

Addressing these research gaps will not only provide critical insights into the comparative phytoremediation potential of *Cannabis sativa* and *Cannabis indica* but also contribute to sustainable remediation practices tailored to the environmental challenges of Western Uttar Pradesh.

4 OBJECTIVES

To address the environmental issue of Lead (Pb) and Cadmium (Cd) contamination in Western U.P.

To evaluate the phytoremediation potential of *Cannabis sativa* and *Cannabis indica* for lead (Pb) and cadmium (Cd) in contaminated soils.

To determine the growth response and health of *Cannabis sativa* and *Cannabis indica* Under lead and cadmium stress.

To identify the potential risks and benefits of using *Cannabis sativa* and *Cannabis indica* for large-scale phytoremediation projects in Western U.P.

To evaluate the impact of phytoremediation on the local ecosystem and soil health in Western U.P.

To study any long-term improvements in soil quality, including pH, organic matter content, and nutrient levels after the remediation process.

5 MATERIALS AND METHODS

Study Area and Plant Selection

The study will be conducted in selected regions of Western Uttar Pradesh (U.P.), known for industrial and agricultural activities contributing to Lead (Pb) and Cadmium (Cd) contamination in soil. Two plant species, *Cannabis sativa* and *Cannabis indica* will be selected for their phytoremediation potential. The plants will be sourced from certified nurseries to ensure genetic purity and uniform growth characteristics.

Experimental Setup

Soil Collection and Preparation

- Contaminated soil samples will be collected from industrial and agricultural sites in Western U.P.
- Collection of samples will be done from different depths(e.g.0-15cm,15-30cm) to account for vertical variation in metal concentration.
- Soil samples will be analyzed for initial concentrations of Pb and Cd using atomic absorption spectroscopy (AAS).
- Comparison of results with permissible limits set by environmental standards (WHO and EPA).
- Regular soil enrichment will be done to maintain the contamination levels at various stages of experimentation.
- Uncontaminated soil samples will be used as control for comparative purposes.

Soil Analysis

- Measurement of soil pH using a digital pH meter in a1:2.5 soil-water ratio.
- Measurement of electrical conductivity (EC) to assess soil salinity using a conductivity meter.

- Soil texture (sand, silt, clay) will be analyzed using the hydrometer method.
- Organic matter content will be assessed using the Walkley-Black method.
- Measurement of the soil's ability to retain cations using ammonium acetate extraction or barium chloride method. Soil cation and anion concentrations will also be analyzed using ion chromatography and spectrophotometric techniques after extracting the ions from soil samples with appropriate solutions, such as ammonium acetate for cations and deionized water or salt solutions for anions.

Plant Cultivation

- Uniform saplings of *Cannabis sativa* and *Cannabis indica* will be transplanted into pots as well as field beds containing contaminated soil and control soil.
- Each species will be grown in minimum five replicates per treatment to ensure statistical reliability.

Field Specification

- A field will be selected that will be contaminated with lead (Pb) and cadmium (Cd), or artificially spike a controlled field with known concentrations of these metals.
- Minimum area: 20 m × 20 m, divided into equal plots of 2m x 2m for *Cannabis sativa* and *Cannabis indica* and control treatments with a buffer zone of 1 m between plots to avoid overcrowding.
- Randomized Block Design(RBD) with replicates for each treatment(*cannabis sativa* plot, *cannabis indica* plot, control plot).

Growth Conditions

- Plants will be grown under controlled conditions maintaining optimal temperature, humidity, and light.
- Regular irrigation will be provided with deionized water to prevent additional contamination.

Lead and Cadmium Treatment

- Pb and Cd concentrations in contaminated soil will be standardized to known levels based on the environmental threshold limits for heavy metals.
- Additional treatments with varying Pb and Cd concentrations (low, medium, and high) will be prepared to study dose-dependent phytoremediation capacity.

Sampling and Analysis

1. **Plant Sampling**
    - Samples of roots, stems, and leaves will be collected at intervals of 30, 60, and 90 days post-transplantation.
    - Plant tissues will be washed thoroughly to remove adhering soil particles.
2. **Soil Sampling**
    - Post-harvest soil samples will be collected to evaluate the reduction in Pb and Cd concentrations.
3. **Heavy Metal Analysis**
    - Plant and soil samples will be digested using acid digestion methods.
    - Pb and Cd concentrations will be quantified using AAS.
4. **Growth and Biomass Assessment**
    - Plant height, leaf area, and biomass yield will be recorded to assess the impact of heavy metals on plant growth.

Statistical Analysis

- Data will be analyzed using statistical software (SPSS).
- ANOVA will be employed to determine significant differences between species, treatments, and time intervals.
- Correlation analysis will evaluate the relationship between metal uptake and plant growth parameters.

Comparative Assessment

- The phytoremediation efficiency of *Cannabis sativa* and *Cannabis indica* will be compared based on:
    - Metal uptake (mg/kg dry weight) in roots and shoots.
    - Reduction of Pb and Cd levels in soil.
    - Plant growth and biomass production.

This methodology will provide insights into the suitability and efficiency of *Cannabis sativa* and *Cannabis indica* for remediating Pb and Cd-contaminated soils in Western U.P.

6 IMPACT OF THE RESEARCH WORK

- The research will provide insights into the efficacy of *Cannabis sativa* and *Cannabis indica* in removing heavy metals like Pb and Cd from contaminated soils, contributing to the detoxification and restoration of polluted lands.
- By identifying which species is more efficient in phytoremediation, the study will promote sustainable soil management practices and reduce the environmental and health risks associated with heavy metal contamination.
- The results will serve as a foundation for localized solutions enhancing the use of *Cannabis* species in managing soil contamination in industrially and agriculturally impacted areas.
- A successful study could lead to the development of cost-effective, eco-friendly strategies for soil remediation, providing an alternative to expensive and less sustainable traditional methods.
- The use of cannabis in phytoremediation may open new agricultural opportunities and generate revenue for local farmers while simultaneously cleaning contaminated land.
- The research will contribute to the growing body of knowledge on phytoremediation, specifically focusing on the comparative effectiveness of different cannabis species in heavy metal removal.
- It will address gaps in understanding the mechanisms of metal uptake, translocation, and accumulation in cannabis plants, providing valuable data for future scientific studies.
- The findings may influence policy frameworks and regulations regarding the cultivation and utilization of cannabis for environmental remediation purposes.
- This research can help shape sustainable agricultural and environmental policies in Western U.P. and other regions with similar challenges.
- By improving the health of contaminated lands, the research can lead to healthier ecosystems and communities, reducing risks associated with exposure to toxic metals.
- It may also raise public awareness about the potential of phytoremediation in addressing environmental challenges and encouraging community participation in sustainable practices.

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